Original Article

Dietary plant sterols supplementation does not alter lipoprotein kinetics in men with the metabolic syndrome

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Dietary plant sterols supplementation has been demonstrated in some studies to lower plasma total and LDL cholesterol in hypercholesterolemic subjects. The cholesterol lowering action of plant sterols remains to be investigated in subjects with the metabolic syndrome. In a randomized, crossover study of 2 x 4 week therapeutic periods with oral supplementation of plant sterols (2 g/day) or placebo, and two weeks placebo wash-out between therapeutic periods, we investigated the effects of dietary plant sterols on lipoprotein metabolism in nine men with the metabolic syndrome. Lipoprotein kinetics were measured using [D_3]-leucine, gas chromatography-mass spectrometry and compartmental modeling. In men with the metabolic syndrome, dietary plant sterols did not have a significant effect on plasma concentrations of total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, apolipoprotein (apo) B, apoA-I or apoA-II. There were no significant changes to VLDL-, IDL-, LDL-apoB or apoA-I fractional catabolic rates and production rates between therapeutic phases. Relative to placebo, plasma campesterol, a marker of cholesterol absorption was significantly increased (2.53 ± 0.35 vs. 4.64 ± 0.59 µg/ml, $p<0.05$), but there was no change in plasma lathosterol, a marker of endogenous cholesterol synthesis. In conclusion, supplementation with plant sterols did not appreciably influence plasma lipid or lipoprotein metabolism in men with the metabolic syndrome. Future studies with larger sample size, stratification to low and high cholesterol absorbers and cholesterol balance studies are warranted.

Key Words: plant sterols, lipoprotein kinetics, dyslipidemia, cholesterol absorption, metabolic syndrome

INTRODUCTION

The metabolic syndrome is a cluster of risk factors for cardiovascular disease that includes visceral obesity, insulin resistance, hypertension and dyslipidemia.[1] There is extensive evidence suggesting that dyslipidemia is a central mediator of this atherogenic condition.[2] The dyslipidemia associated with the metabolic syndrome is characterized by elevated plasma triglycerides, decreased HDL cholesterol and high concentrations of apolipoprotein (apo) B-containing lipoproteins, as well as increased flux of non-esterified free fatty acids (NEFA) to the liver.[3]

National guidelines recommend weight loss as the first step in the management of dyslipidemia and prevention of coronary heart disease morbidity and mortality.[4] In the absence of weight loss, dietary changes, which include reduction in fat intake and the addition of adjuncts such as plant sterols or stanols to reduce cholesterol absorption, can be applied.[5] Plant sterols and cholesterol although differing structurally retain sufficient similarity to compete for sites of sterol absorption in the intestines.[6] Plant sterols compete with cholesterol for incorporation into intraluminal intestinal micelles, thus reducing cholesterol uptake by the brush border membrane.[7]

Clinical trials, predominantly in hypercholesterolemic subjects have supported the benefit of dietary supplementation with plant sterols.[8,9] These studies demonstrated that the addition of 2 to 3 g/day of plant sterol esters and plant stanol esters (hydrogenated form of plant sterols esters) can reduce total cholesterol and LDL cholesterol by up to 15%.[10] This could translate to a favorable 25% reduction in coronary artery disease.[11] Furthermore, two studies employing radioisotopes in hypercholesterolemic patients with type 2 diabetes have shown that plant sterols (specifically sitostanol) can decrease LDL apoB production with no apparent change to LDL apoB catabolism.[12,13]

In this study, we sought to determine whether plant sterols are effective in lowering LDL cholesterol in subjects with the metabolic syndrome and its effect on lipoprotein metabolism, specifically VLDL-, intermediate-density lipoprotein- (IDL) and LDL-apoB and HDL apoA-I. On the basis that metabolic syndrome subjects have high absolute cholesterol absorption rates despite having low fractional

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cholesterol absorption rates, we hypothesized that dietary plant sterols supplementation in this group would reduce LDL cholesterol concentration and apoB transport rate.

METHODS AND METHODS

Subjects

Nine Caucasian men with the metabolic syndrome were recruited through the distribution of flyers and newspaper advertisements. The selection criteria was as follows: age range of 45 – 70 years, BMI ≥ 30 kg/m^2 or waist:hip ratio > 0.9, triglycerides > 1.7 mmol/L, HDL cholesterol <1.10 mmol/L and fasting glucose > 6.1 mmol/L. Participants were assessed by on-site physicians and those with diabetes mellitus, history of cardiovascular disease, genetic or secondary lipid disorders and consuming lipid-modifying agents were excluded from the study. The study was approved by the Alfred Hospital Human Ethics Committee, and all subjects provided written consent.

Study Design and Clinical Protocol

The study was a randomized, single-blind, cross-over of 2 x 4 weeks therapeutic periods with non-plant sterol enriched (placebo) or plant sterol-enriched foods within a breakfast cereal and margarine (Goodman Fielder Pty Ltd, 2 g/day plant sterols, in the ester form) with two weeks wash-out between therapeutic periods. Subjects entered a 2-week run-in diet-stabilizing and weight maintenance period prior to randomization. Subjects were instructed to consume a sachet of cereal and 1.5 tablespoons of margarine at breakfast. A further 1.5 tablespoons of margarine were consumed in a sandwich at lunch. In addition, two eggs (approximately 450 mg cholesterol) were consumed daily, one at breakfast and one at lunch throughout the study; this ensured a more steady consumption of cholesterol than would be possible with lower-cholesterol content foods in a free-living population. By increasing cholesterol intake, a plateau effect on LDL cholesterol is achieved and hence, a steady-state for cholesterol metabolism is probable.[14] Subjects were advised against consuming self-purchased plant sterols-enriched products and to maintain current diet and physical activity for the duration of the study.

At the end of each 4-week treatment period, lipoprotein turnover studies employing stable isotope methodologies were performed. All subjects were admitted to the metabolic ward in the morning following a 12-hour overnight fast. They were studied in a semi-recumbent position and allowed only water to drink for the next 10 hours. Venous blood was collected for biochemical measurements. Arterial blood pressure was recorded after three minutes in the supine position using a Dinamap1846 SX/P monitor (Critikon, Tampa, FL).

A single bolus injection of [D_3]-leucine (4mg/kg) was administered intravenously into an antecubital vein via a Teflon cannula. The [D_3]-leucine (in powder form) was supplied from Cambridge Isotope, MA, USA and prepared as sterile solution for injection by the Pharmacy at Royal Perth Hospital. Blood samples were collected at baseline and at 5, 20, 30 and 45 minutes and at 1, 2, 3, 4, 6, 8, 10 hours post-injection from the opposite arm. Subjects were then provided a meal and allowed home. Additional fasting blood samples were collected on the four mornings of the same week (24, 48, 72 and 96 hours). Subjects continued to consume eggs, cereal and margarine for breakfast and lunch during this period, with the exception of the 10-hour study day; on this day, the first and only intake of the foods (egg, cereal and margarine) were consumed in the evening after the collection of the 10-hour blood sample.

Blood samples were collected into EDTA tubes, kept on ice and spun at 312200 g for 15 minutes at 4°C. Plasma samples collected were stored in -80°C prior to lipoprotein sub-fraction isolation.

Diet Records

Dietary intake was monitored using Victorian Anti-Cancer Foundation diet assessment books (food frequency) during each intervention period to determine compliance and assess nutrient intake. Compliance with margarine intervention was determined by measuring the weight of margarine consumed at the end of each therapeutic period. The number of cereal packets that were used was recorded.

Isolation and measurement of isotopic enrichment of apoB and HDL apoA-I

ApoB. The isolation of VLDL-, IDL- and LDL- apoB was performed as previously described.[15] In brief, VLDL, IDL and LDL fractions were isolated from 2 mL plasma by sequential ultracentrifugation (Optima XL-100K; Beckman Coulter, Fullerton, Australia) at densities of 1.006, 1.019 and 1.063 g/mL, respectively; precipitated by isopropanol, delipidated, hydrolyzed and derivatized using the oxazolinone derivative. Plasma-free leucine was isolated by cation-exchange chromatography using AG 50 W-X8 resin (Biorad, Richmond, CA) following removal of plasma protein with 60% perchloric acid. The isotopic enrichment was determined using gas chromatography mass spectrometry (GCMS) with selected ion monitoring of samples at a mass-to-charge ratio (m/z) of 212 and 209 and negative ion chemical ionization. Tracer-to-tracee ratios were derived from isotopic ratios of each sample.

ApoA-I. The HDL fraction was isolated from sequential ultracentrifugation of 2 mL plasma adjusted to a density of 1.48 g/mL.[16] ApoA-I was isolated using SDS-PAGE, transferred onto a polyvinylidine fluoride membrane, excised from the membrane and hydrolyzed with 200 µL 6 M HCL at 110°C for 16 hours, dried down and derivatized as previously described. Enrichment of [D_3]-leucine was measured as described above.

Quantification of apoB

Plasma samples were combined and four pooled fractions of VLDL, IDL and LDL from each treatment period were isolated as described above. ApoB was isolated using isopropanol and quantified with the Lowry method.[17]

Serum lipids, lipoproteins and other analyses

Total cholesterol and triglyceride concentrations were measured using a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland) using enzymatic kits (Hoffman-La Roche Diagnostica, Basel, Switzerland) and standard control sera. Plasma HDL cholesterol

concentrations were measured after precipitation of apoB-containing lipoprotein by PEG 6000. The following modification of the Friedewald equation for molar concentrations was used to calculate LDL cholesterol (in mmol/L): LDL cholesterol = total cholesterol – (triglyceride/2.18) – HDL cholesterol. Total plasma apoB, apoA-I and apoA-II concentrations were determined using immunonephrelometry (Dade Behring BN_2 Nephelometer). Plasma insulin was measured by radioimmunoassay (DiaSorini, Saluggia, Italy) and glucose analyzed on a Hitachi 917 Biochemical Analyzer. Insulin resistance was estimated using the homeostasis model assessment (HOMA) score, which was defined as plasma glucose (mmol/L x plasma insulin (mmol/L)/22.5.[18] All other routine biochemistry and hematology was performed at an accredited pathology laboratory (CV < 10% for all analyses).

Plasma Plant sterols and Lathosterol

Plasma plant sterols concentrations were measured according to the methods described by Wolthers et al.[19] In brief, 400 µL of plasma sample was saponified with 400 µL of 33% KOH at 60°C for 30 minutes, cooled, and extracted with hexane. The extract was evaporated to dryness with a stream of nitrogen, and the plant sterols were derivatized by treatment with 150 µL of SyLON BTZ (Supelco) for 30 min at 80°C. The silyl derivatives of the plant sterols were extracted into hexane and concentrated with a stream of nitrogen to 50 µL, and a 1 µL aliquot was injected onto the GC column (split ratio of 1:10). The gas chromatograph consisted of a DANI 6500 instrument equipped with a split/splitless injector and a flame ionization detector coupled to a DELTA computerized chromatography data system. The injector, detector, and oven temperatures were set at 275, 275, and 280°C, respectively. The capillary column used was a 60 m x 0.22 mm BPX5 (SGE Australia P/l). Plasma plant sterols concentrations were calculated from the standard curves using the ratio of the plant sterols peak area to the peak area of the internal standard (5β-cholestan-3α-ol). The pure internal standard and lathosterol, campesterol, and sitosterol reference samples were obtained from Sigma Chemical Co. (St. Louis, MO) (CV< 10% for all analyses).

Kinetic Analyses

The fractional catabolic rate (FCR) of VLDL-, IDL- and LDL-apoB and HDL apoA-I were estimated using multi-compartmental models (SAAM Institute, Seattle, WA) as described previously.[20] The SAAM II program fits the model to the observed tracer data using a weighted-least-squares approach to estimate lipoprotein FCRs. Lipoprotein production rates (PR) (mg/kg/day) were then calculated as a formula of lipoprotein concentration (mg/L) × plasma volume (0.045 L/kg × body weight) × FCR (pools/day).

Statistical analysis

Data are reported as mean ± S.E.M. Skewed variables were logarithmically transformed where appropriate. Comparisons between therapeutic phases were carried out using paired t test. Statistical significance was defined at $p<0.05$. All data were analyzed using SPSS software (SPSS 11.5, Chicago, USA). This study was designed to have an 80% power to detect a 15 – 20% change in plasma LDL cholesterol concentration and apoB transport rate between placebo and plant sterols treatment phases with an α-error of 5%. Any smaller effects would be missed.

RESULTS

Nine men with the metabolic syndrome recruited for this study completed the 12-week trial. Dietary compliance assessed as percentage of supplements consumed was as follows: margarine, 91 and 98%; egg, 97 and 99%; and cereal, 96 and 98% for placebo and plant sterols treatment respectively. There were no significant changes between the placebo and plant sterols treatment for total fat (102 ± 13.2 vs. 111 ± 7.9 g/day, $p= 0.394$), saturated fat (38.8 ± 5.81 vs. 41.0 ± 3.29 g/day, $p = 0.633$), poly-unsaturated fat (17.8 ± 2.27 vs. 20.7 ± 1.84 g/day, $p = 0.225$), mono-saturated fat (35.6 ± 4.95 vs. 39.2 ± 3.17 g/day, $p = 0.392$), protein (111 ± 11.4 vs. 123 ± 10.8 g/day, $p = 0.370$), carbohydrate (272 ± 31.7 vs. 267 ± 19.4 g/day, $p = 0.852$), fiber (29.2 ± 3.4 vs. 30.7 ± 3.1 g/day, $p= 0.647$), cholesterol (459 ± 61.4 vs. 492 ± 66.6 mg/day, $p = 0.390$), or energy (10186 ± 1125 vs. 10633 ± 701 kJ/day, $p = 0.647$).

Table 1 shows the clinical characteristics of the subjects at entry into the study. The mean age of the subjects was 60 ± 3 years. Subjects were overweight or obese with central adiposity, insulin resistant and dyslipidemic.

Table 2 shows the clinical characteristics of the subjects after treatment with placebo or plant sterols. There were no significant changes in weight, BMI, waist:hip ratio, HOMA score or systolic and diastolic blood pressure associated with plant sterols treatment.

Table 3 compares the plasma lipids, lipoproteins and apolipoproteins concentrations between placebo or plant sterols treatments. There were no significant changes to total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol apoB, apoA-I or apoA-II concentrations following plant sterols treatment.

Table 4 shows the results for plasma plant sterols and lathosterol (a marker of endogenous cholesterol synthesis) after treatment with placebo or plant sterols. Plasma lathosterol, desmosterol and stigmasterol were not statistically different between treatments. Campesterol and sitosterol (markers of sterol absorption) increased significantly by 70% and 44%, respectively, during plant sterols

Table 1. Clinical and biochemical characteristics of subjects studied

Variable	Mean	S.D.	Range
Age (years)	60.1	8.51	50 – 70
Weight (kg)	110	19.1	78 – 136
BMI (kg/m^2)	35.2	5.09	27 – 43
Waist:Hip Ratio	1.00	0.09	0.9 – 1.2
Fasting Glucose (mmol/L)	5.66	0.49	5.2 – 6.8
Total Cholesterol (mmol/L)	5.53	1.45	4.1 – 8.9
Triglyceride (mmol/L)	2.27	0.75	1.2 – 3.6
LDL cholesterol (mmol/L)	3.51	1.26	2.5 – 6.7
HDL cholesterol (mmol/L)	1.02	0.23	0.5 – 1.3

Mean ± S.D.

Table 2. Clinical and biochemical characteristics of subjects with or without plant sterols supplementation

Variable	Placebo	Plant Sterols	*p*-value
Weight (kg)	111 ± 7.74	111 ± 6.46	0.080
BMI (kg/m^2)	35.5 ± 1.54	35.3 ± 1.68	0.083
Waist:Hip Ratio	1.00 ± 0.04	1.01 ± 0.03	0.257
Fasting Glucose (mmol/L)	5.3 ± 0.12	5.4 ± 0.11	0.308
Insulin (mmol/L)	20.3 ± 2.67	20.3 ± 4.78	0.762
HOMA Score	4.8 ± 0.65	4.9 ± 1.25	0.713
Systolic (mmHg)	127 ± 5.39	127 ± 5.32	0.882
Diastolic (mmHg)	75 ± 2.96	77 ± 3.10	0.238
Heart Rate	70 ± 4.06	69 ± 4.87	0.613
Pulse Pressure	53 ± 4.39	49 ± 4.63	0.271

Mean ± S.E.M.

Table 3. Effects of plant sterols supplementation on plasma lipids, lipoproteins and apolipoproteins concentrations

Variable	Placebo	Plant Sterols	*p*-value
Total cholesterol (mmol/L)	5.42 ± 0.46	5.27 ± 0.48	0.313
Triglyceride (mmol/L)	1.83 ± 0.22	1.93 ± 0.24	0.588
LDL cholesterol (mmol/L)	3.42 ± 0.42	3.20 ± 0.44	0.286
HDL cholesterol (mmol/L)	1.16 ± 0.06	1.19 ± 0.11	0.563
Total apoB mass (g/L)	1.21 ± 0.12	1.09 ± 0.12	0.163
ApoA-I mass (g/L)	1.34 ± 0.06	1.38 ± 0.07	0.405
ApoA-II mass (g/L)	0.32 ± 0.02	0.33 ± 0.02	0.363

Mean ± S.E.M.

Table 4. Effects of plant sterols supplementation on lathosterol and plasma plant sterols concentrations

Variable	Placebo	Plant Sterols	*p*-value
Lathosterol (µg/ml)	4.08 ± 0.30	4.16 ± 0.32	0.818
Desmosterol (µg/ml)	1.17 ± 0.14	1.40 ± 0.27	0.459
Stigmatosterol (µg/ml)	0.41 ± 0.11	0.44 ± 0.13	0.608
Campesterol (µg/ml)	2.58 ± 1.18	4.66 ± 0.66	0.001
Sitosterol (µg/ml)	1.83 ± 0.23	2.64 ± 0.42	0.008

Mean ± S.E.M.; Footnote: We also compared plasma lathosterol, campesterol and sitosterol concentrations between our study population (not on plant sterols treatment) with a group of healthy normolipidemic lean men of comparable age to examine whether our subjects had relatively higher *de* novo cholesterol synthesis and/or lower cholesterol absorption. Compared to the lean individuals, our subjects had a significantly higher concentration of plasma lathosterol (2.87 ± 0.3 vs. 4.07 ± 0.3 µg/ml, $p < 0.05$) and significantly lower campesterol and sitosterol concentrations (3.79 ± 0.4 vs. 2.58 ± 0.4 µg/ml and 3.82 ± 0.4 vs. 1.84 ± 0.3 µg/ml, respectively, $p<0.05$). Lathosterol to campesterol and lathosterol to sitosterol ratios were both significantly lower in the lean individuals compared to our study subjects (1.75 ± 0.2 vs. 0.87 ± 0.1 and 2.38 ± 0.2 vs. 0.87 ± 0.1, respectively, $p<0.001$).

Table 5. The effects of plant sterols supplementation on VLDL-, IDL- and LDL-apoB and HDL apoA-I pool size, production rate (PR) and fractional catabolic rate (FCR)

Parameters	Placebo	Plant Sterols	*p*-value
Pool size (mg)			
VLDL apoB	332 ± 30	384 ± 55	0.155
IDL apoB	150 ± 18	175 ± 32	0.322
LDL apoB	2774 ± 163	2615 ± 96	0.271
HDL apoA-I	6681 ± 431	6825 ± 440	0.405
Fractional Catabolic Rate (pools/day)			
VLDL apoB	4.29 ± 0.59	3.52 ± 0.32	0.122
IDL apoB	6.06 ± 0.68	6.94 ± 0.52	0.268
LDL apoB	0.34 ± 0.04	0.30 ± 0.03	0.326
HDL apoA-I	0.30 ± 0.01	0.33 ± 0.02	0.419
Production Rate (mg/kg/day)			
VLDL apoB	12.0 ± 0.80	11.4 ± 0.94	0.833
IDL apoB	7.59 ± 0.43	10.8 ± 1.98	0.689
LDL apoB	8.13 ± 0.57	7.09 ± 0.65	0.163
HDL apoA-I	18.2 ± 1.01	20.2 ± 1.73	0.246

Mean ± S.E.M.

treatment.

Table 5 shows the VLDL-, IDL- and LDL-apoB, and HDL apoA-I pool sizes and kinetic parameters during placebo and plant sterols treatment periods. There were no significant changes to VLDL-, IDL- and LDL-apoB of HDL apoA-I pool sizes, PRs or FCRs.

Figure 1 shows the compartmental model used to estimate VLDL-, IDL- and LDL-apoB, and HDL apoA-I kinetic parameters.[20]

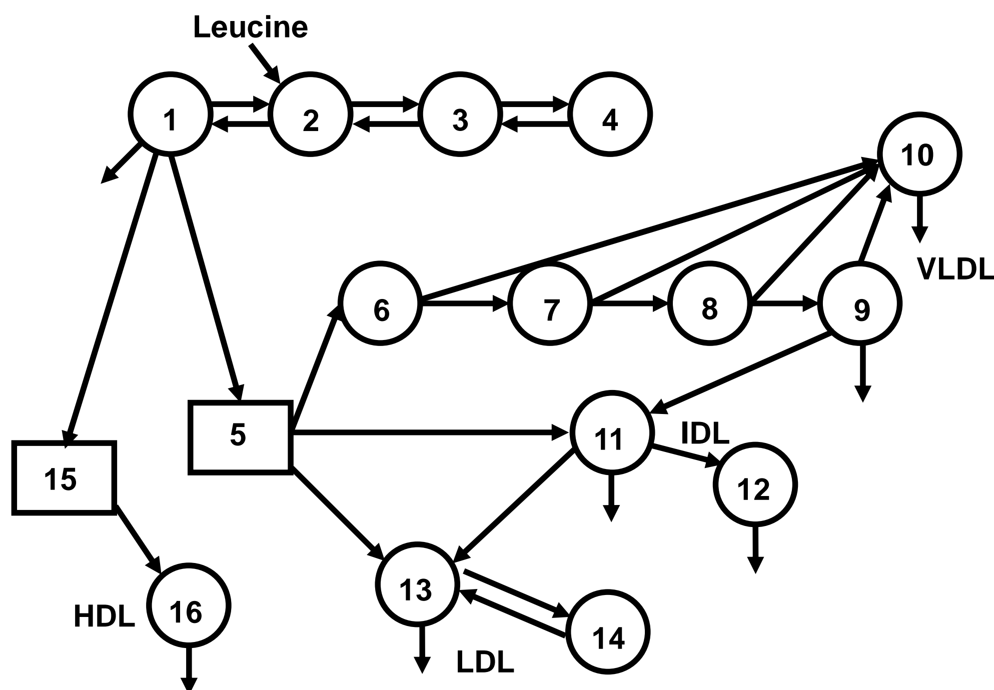
DISCUSSION

Our principal result was that dietary plant sterols supplementation did not appreciably alter apoB-100 and HDL apoA-I kinetics and as a consequence, the concentrations of total cholesterol, LDL cholesterol, triglycerides and HDL cholesterol remained unchanged. Our study extends a recent negative report from a larger study with hypercholesterolemic subjects [21] by measuring apolipoprotein kinetics and studying the metabolic syndrome.

The cholesterol lowering effects of plant sterols were previously demonstrated in mild hypercholesterolemic subjects and type 2 diabetics with hypercholesterolemia [13,22]. Studies in post-menopausal women also demonstrated beneficial effects of the addition of plant sterols to the daily diet.[23] In those studies, plant sterols significantly reduced serum and LDL cholesterol. However, there are some studies that have reported the lack of efficacy of plant sterols. In a long term study, Brink and Hendriks [24] showed that consumption of plant sterol esters enriched spread for one year did not lower total cholesterol concentrations in men, although a significant reduction (5%) in LDL cholesterol was shown. Another study in normal to mild hypercholesterolemic subjects, which tested up to 9 g/day of plant sterols also, did not find significant total or LDL cholesterol lowering.[25] A recent study reported that a single morning dose of standard and novel plant sterols preparations for four weeks did not improve lipid concentrations in 30 subjects with mild to moderate hypercholesterolemia [21]. On the other hand, it is worth noting that even modest consumption of plant sterols naturally occurring in oilseeds (such as corn and wheatgerm) is inversely correlated with serum cholesterol levels.[26,27]

The present results need to be considered in the context of several reports showing reduced responsiveness by subjects with insulin resistance, including the metabolic syndrome, to dietary cholesterol changes.[28-30] Subjects who are insulin sensitive respond to changes in dietary cholesterol with greater movements in their LDL cholesterol concentration than in insulin resistant subjects regardless of their degree of obesity.[31] In addition, insulin resistant subjects exhibited lower cholesterol absorption efficiency and high cholesterol synthesis.[29] When compared to a population of lean normolipidemic subjects previously studied, individuals in this study have significantly lower cholesterol absorption and higher rates of *de novo* cholesterol synthesis (refer to Footnotes, Table 4). This may contribute to an altered intestinal cholesterol pool and/or the absorption mechanisms of the intestinal

Figure 1. Compartmental model describing apoB and HDL apoA-I tracer kinetics



Footnote: Leucine tracer is injected into plasma, represented by compartment 2. Compartments 1, 3 and 4 represent nonplasma leucine compartments. ***ApoB:*** Compartment 5 represents an intrahepatic pool that accounts for the time associated with the synthesis, assembly and secretion of apoB in VLDL, IDL and LDL fractions. VLDL apoB is described by 5 compartments (6 – 10). VLDL apoB is converted to IDL (compartment 11) or cleared directly from plasma. IDL apoB is represented by 2 compartments (11 and 12) and is either cleared from plasma, or converted to LDL (compartment 13). LDL apoB is cleared from this compartment (13) and exchanges with an extravascular LDL pool, compartment 14. ***HDL apoA-I:*** Compartment 15 represents an intrahepatic pool that accounts for the time associated with the synthesis, assembly and secretion of apoA-I in HDL fraction. HDL apoA-I is represented as a single plasma compartment, compartment 16, and is cleared from this compartment.

mucosa [30,32] and the lack of response to plant sterols supplement observed in our subjects.

In this study, supplementing with plant sterols clearly increased the plasma campesterol concentration indicating that exposure of the intestine to the sterol esters was of similar magnitude as reported in other studies.[33,34] On the other hand, cholesterol synthesis, as indicated by plasma lathosterol concentration, did not change significantly. This is consistent with a lesser effect on cholesterol synthesis in the liver that may in turn explain the absence of changes in apoB-containing lipoprotein metabolism. Effects on lathosterol do not occur consistently with modest changes in cholesterol absorption.[35]

Endogenous lipoprotein metabolism is in part, regulated by the delivery of cholesterol and triglycerides to the liver.[36] Dietary cholesterol and triglycerides are present in mixed micelles and absorbed into the luminal wall, packaged into large chylomicron particles and stored in the liver as cholesterol esters or exported as lipoproteins into plasma. An important but unresolved question is whether the dyslipidemia observed in insulin resistant states, including the metabolic syndrome is primarily attributable to increased cholesterolgenesis or excess flux of NEFA to the liver. Subjects with the metabolic syndrome have increased concentration of NEFA that drive triglyceride synthesis and increases VLDL secretion.[37] If NEFA is driving the overproduction of apoB, inhibition of cholesterol absorption by plant sterols alone may not be sufficient in addressing the dyslipidemia of the metabolic syndrome. This may in part, explain the lack of response of our subjects to plant sterols supplement.

Diurnal periodicity in human cholesterol synthesis may influence the absorption of cholesterol from the intestine. Jones and Schoeller [38] proposed that *in vivo* cholesterol synthesis varies with a circadian rhythm. Their finding suggests that endogenous cholesterol synthesis was higher during the morning, diminishes in the afternoon and rises again at night. If hepatic cholesterol production was relatively high (coinciding with low cholesterol absorption) during the period of plant sterols treatment then the effect of the plant sterols may have been minimized. This is, plant sterols further reduced cholesterol absorption from a pool that was already poorly absorbed.

In this study, plant sterol supplement did not influence HDL metabolism. While dietary fat and cholesterol intake may up-regulate HDL apoA-I mRNA expression and synthesis,[39] the degree of effect of plant sterols on cholesterol absorption was insufficient to influence HDL metabolism.

We acknowledge the limitations in our study. Our analysis was based on biochemical markers of cholesterol absorption and synthesis and not definitive measures of cholesterol absorption and synthesis. Nonetheless, plasma campesterol and lathosterol quantification have been shown to be strongly correlated with direct measures of cholesterol absorption and synthesis, respectively.[40] This study was powered to detect a 15 - 20% treatment effect on plasma LDL cholesterol concentration and apoB transport rate. Hence, with the present sample size, smaller effects of plant sterols supplementation might have been missed. In the normal population, gastrointestinal absorption at low to moderate intakes of cholesterol varies between 25 – 80%, implying genetic influence to the absorption pathways.[41,42] Common sequence variations such as in the *ABCG8* gene also influence the absorption of plant sterols in normal populations.[43]

In conclusion, there appears to be no significant effect of dietary supplementation with plant sterols (2 g/day) in plasma lipoprotein concentration and kinetics subjects with the metabolic syndrome. Persistent high rates of de novo cholesterol synthesis together with the compensatory decrease in fractional cholesterol absorption that occurs in obese individuals with the metabolic syndrome may explain the failure of attempting to reduce cholesterol absorption further. Future studies with larger sample sizes, stratification to low and high-cholesterol absorbers as well as more direct estimates of cholesterol balance across the intestines and liver are warranted to better the potential of plant sterols treatment to regulate cholesterol absorption.

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AUTHOR DISCLOSURES

Esther MM Ooi, Gerald F Watts, P Hugh R Barrett, Dick C Chan, Peter M Clifton, Juying Ji and Paul J Nestel, disclose no conflicts of interest other than those in the ACKNOWLEDGEMENTS.

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Original Article

Dietary plant sterols supplementation does not alter lipoprotein kinetics in men with the metabolic syndrome

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補充膳食植物固醇不影響代謝症候群男性之脂蛋白動力學

部分研究已證實高膽固醇血症患者補充膳食植物固醇，可以降低其血漿總膽固醇及 LDL 膽固醇濃度。對代謝症候群患者，植物固醇降低膽固醇的作用仍待研究。我們設計一個隨機交叉，處置時間為 2x4 週的研究，探討膳食固醇對九名有代謝症候群男性脂蛋白代謝之影響。給予口服植物固醇補充劑(2g/天)或是安慰劑，兩種處置期間的休閒期兩週給予安慰劑。採用[D_3]-leucine、氣相層析質譜儀以及隔式模型測量脂蛋白動力學。對患有代謝症候群的男性而言，膳食固醇對於血漿總膽固醇、三酸甘油酯、LDL 膽固醇、HDL 膽固醇、輔蛋白(apo)B、輔蛋白 A-I 或 A-II 之濃度沒有顯著的影響。VLDL-、IDL-、LDL-輔蛋白 B 或輔蛋白 A-I 部分的分解率及生產率在兩種不同處置間並沒有顯著改變。相對於安慰劑組，膽固醇吸收標記-血漿 campesterol 顯著增加(2.53 ± 0.35 vs. 4.64 ± 0.59 μg/ml, $p<0.05$)，但是內生性膽固醇合成標記-血漿 lathosterol 則沒有改變。總之，補充植物固醇對於代謝症候群男性患者之血漿脂質或是脂蛋白代謝並沒有很大的影響。未來研究應有較大的樣本，並且作低、高膽固醇吸收者分層，以及膽固醇平衡研究。

關鍵字：植物固醇、脂蛋白動力學、血脂異常、膽固醇吸收、代謝症候群。